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A STUDY OF THE VARIOUS CHANGES WHICH OCCUR IN THE TISSUES IN ACUTE DIPHTHERITIC TOXÆMIA, MORE ESPECIALLY IN REFERENCE TO "ACUTE CARDIAC FAILURE."

(From the Louis Jenner Clinical Laboratory, St. Thomas's Hospital.)

BY LEONARD S. DUDGEON, M.R.C.P.LOND.

Bacteriologist to St. Thomas's Hospital and Joint Lecturer on Pathology in the Medical School; Director of the Louis Jenner Clinical Laboratory and of the Pathological Laboratories, St. Thomas's Hospital.

THE cost of this research was defrayed through the munificence of Lady Jenner, who has established a scholarship in pathological research at St. Thomas's Hospital, in memory of her son, Louis Jenner. Nothing has been more gratifying to me than to have been able to carry out this investigation in the laboratory where, at one time, I had the advantage of being an assistant to my friend, Dr. Louis Jenner.

INTRODUCTION.

At the suggestion of Dr. Foord Caiger, I have made an investigation of the various changes which occur in the viscera, nerves and muscles, in sixteen cases of acute diphtheritic toxæmia. A large amount of experimental work has also been done with the filtered toxins of the diphtheria bacillus. By comparing the results of the pathological investigations in the human cases with those which have been found in the inoculated animals, I have been able to form a definite idea of the changes which occur.

Bristowe [5] examined the heart muscle in a case which was clinically malignant hæmorrhagic diphtheria, and found

it to be extremely fatty, and the adrenal glands to be hæmorrhagic. The kidneys also showed fatty change. He mentioned that in his opinion the heart muscle is usually normal in diphtheria, but in this instance the fatty change was very well marked.

Flexner [7], in his valuable monograph on the pathology of the toxalbumin intoxications, remarks that fatty metamorphosis is one of the most common pathological conditions found in the myocardium in diphtheria. I can do no better than quote his own words: "For its detection we have employed the frozen section only. It was rarely absent, and was encountered, more especially in those cases which terminated fatally, within short periods following inoculation." He also draws attention to the swelling and elongation of the nuclei and alteration in their shape, the fibres in the meantime showing little change. As the changes in the nuclei become more marked the substance of the fibres has disappeared, or has taken on a swollen and attenuated aspect. Pathological alterations in the interstitial tissue were wanting. Vascular changes were noted.

The chief lesion of the adrenal gland was congestion and hæmorrhage. The medulla, more especially, was full of blood. Necrosis of the tissue cells, such as has been met with in the liver, with invasion of the necrotic areas with polymorphonuclear cells, was commonly found to be present. Fatty metamorphosis of the liver cells was common.

The researches by Flexner were carried out with filtered sterile cultures of the bacillus of diphtheria, and cultures freed from bacteria which had been killed with chloroform.

These observations of Flexner's are of the utmost importance, and form a very considerable addition to our knowledge of this subject; but there is no doubt, however, that many valuable points concerned in diphtheritic toxæmia are omitted.

Vincent reported a case of heart paralysis in diphtheria in which the cardiac plexus showed an atrophy of nerve fibres and myelin sheaths. The pneumogastric nerve and medulla are said to have been normal. (Flexner.)

Müller [13] describes abundance of fat in the splenic

pulp and follicles, and states that it is found to be most abundant in the neighbourhood of the necrotic foci met with in this viscus. He also refers to invasion of the tissues with polynuclear leucocytes.

Bezançon [2] refers to the cloudy swelling, parenchymatous degeneration and fatty change which have been found in the liver, especially in septic cases.

Stanley [17], in an interesting paper published in the *British Medical Journal* in 1903, states that in his opinion the prime factor for the cause of death in diphtheria and beri-beri is the cardiac muscle. He is most emphatic on this point. He says, "To attribute heart failure in beri-beri and diphtheria to a necrosis of the vagus is alike unnecessary, inadequate, and unproved." He, however, concludes this very important statement with some interesting remarks, to which I will refer again later. "In beri-beri and diphtheria the cardiac change is parenchymatous degeneration of the heart muscle." "Sometimes fatty degeneration only is seen and appears to be a later stage of the granular albuminous degeneration." "While the granular degeneration is usually general, the fatty degeneration is more often patchy."

He also adds that, "The heart muscle degeneration takes place as a rule before skeletal muscle degeneration, and is the result probably of direct action of the toxin, and not a secondary result of nerve change."

Hamilton Wright [20], however, adopts quite a different view to explain the cause of rapid death which occurs in acute beri-beri. He says: "In acute pernicious beri-beri the vagal cardiac terminations bear the brunt of the poison, and this, together with poisoning of the accelerator termination, soon leads to cardiac exhaustion and death." He, however, mentions that the muscle fibres of the heart in acute beri-beri are found to be extremely fatty, especially on the right side and in the papillary muscle.

Poynton [15] investigated the heart muscle in eighteen cases of rheumatic cardiac disease, four cases of diphtheria, one of chorea, and the heart muscle of a rabbit in which septicæmia had been produced by means of "staphylococci."

He gives a typical example of each disease to illustrate the most important points.

Thus in a child, aged 5, who died on the seventeenth day of disease from progressive cardiac failure, he found the following changes: The transverse striation of the heart muscle was lost in many places, while some of the fibres showed very marked longitudinal striation. Irregular fatty change occurred throughout the cardiac muscle; some of the fat droplets were of very large size. The muscle nuclei showed abnormal staining reactions, and there was some increase of the cellular elements in the interstitial tissue. The pericardium was not inflamed.

In a boy of 19 years of age, who died rapidly in a third attack of rheumatic fever, some acute pericarditis was found at the autopsy, together with acute endocarditis of the aortic valve and chronic mitral disease. Here extreme fatty change was observed throughout the cardiac muscle. Some interstitial inflammation was present, as was only to be expected.

The heart muscle of the rabbit, which had died from "staphylococcic pyæmia" on the sixth day of illness, showed foci of inflammation throughout its substance, and definite patchy fatty changes.

The heart muscle was also examined in a case of chorea, and early fatty change was noticed in some of the fibres.

Villy [18] examined the stomach in fifteen cases of diphtheria, and found fatty degeneration of the gland cells to be a well-marked feature. In some examples the fatty change affected the whole of the glandular tissue, in other instances it appeared to be more patchy in character. The cases from which the affected stomachs were obtained had been fed during the last few days of life by nutrient enemata. Collections of leucocytes in the mucosa and submucosa, chiefly of the lymphocyte variety, were a constant feature. Fatty change in the heart muscle was also found to be present in his cases, so that this investigator arrived at the conclusion that the cause of the vomiting in diphtheria and the cardiac failure depended upon the microscopical changes found in the stomach wall and heart muscle.

Millard and Regaud [12], from a series of experimental investigations on diphtheritic toxæmia in animals, found that leucocytes are present in large numbers in the interstitial tissue of the various viscera; that the foci of fatty degeneration which occur are composed of leucocytes; that the leucocytes absorb the muscular *débris* and especially the exudates, and that the muscular lesion provokes the leucocytosis.

Andrewes [1] states that: "The muscular tissue of the heart may be in a condition of fatty degeneration which varies to a greater or less degree, but is frequently absent." He also mentions that the cardiac muscle may present advanced fatty change affecting all the fibres, or it may be patchy in character. He considers that the diphtheritic toxins are especially nerve poisons. It should be stated, however, that Andrewes maintains that, in cases of cardiac syncope, the cause of the condition is due to a direct action of the toxin on the cardiac muscle.

Gee [1], in his clinical description of diphtheria in *Allbutt's Medicine*, says: "The cause of the heart failure is interstitial myocarditis with granular and hyaline degeneration of the muscular fibres."

Mott [1], referring to fatty degeneration, says: "From a large experience in the examination of hearts, I am certain that fatty degeneration may be overlooked unless a microscopical examination be made after staining with osmic acid. Thus, in a case of fatal syncope occurring in diphtheritic paralysis, I found the organ extremely degenerated; yet the heart had been passed as normal on macroscopical examination." "I have observed intense fatty degeneration of the heart in a case of diphtheritic paralysis, but I could find no degeneration of the nerve trunks."

Romberg [16] examined the heart muscle in eight fatal cases of diphtheria, in five of which there was *pericarditis*, and in three *endocarditis*. Patchy change was found scattered throughout the cardiac muscle. Central vacuolation of the individual fibre was also noted. The most marked fatty change was noticed in the fibres beneath the endo- and peri-cardium. Interstitial inflammation was constantly present.

Hesse [8] made an examination of twenty-nine cases of diphtheria, in twenty-five of which interstitial myocarditis was present, and in four it was well marked. The change was noticeable in the first week, and more pronounced later. Leucocytic infiltration was believed to be due to escape of the leucocytes into the tissues, owing to injury of the capillary walls by the toxin.

Papkow (quoted by Welch and Schamberg [6]) found extensive fragmentation of the muscle fibres as early as the third and fourth days.

Councilman, Mallory and Pearce [6] found fatty degeneration, varying in extent, in thirty-six out of sixty cases examined, but only forty of these were recent cases, and of this number twenty-nine showed fatty degeneration. In some instances the fatty change was diffuse, in others of the patchy type. It appeared to accompany or precede the more advanced forms of degeneration which lead to complete destruction of the muscular tissue. The muscular elements became swollen, broken and converted into hyaline masses. Vacuolation, fragmentation and fracture of the degenerated fibres were often seen. Proliferation of the cells of the interior of the blood-vessels was found throughout the body. Two kinds of interstitial change were also noted :—

(1) Local collection of plasma and lymphoid cells.

(2) Interstitial change, secondary to muscle degeneration, which may lead to fibrosis.

These observers also state that when fatty degeneration of the heart is present, similar changes will be found in the skeletal muscle. They failed to find any gross lesion in the adrenal gland.

Welch and Flexner [19] found congestion, hæmorrhage, and focal necrosis in experimental diphtheria, but not in the human subject.

Marked parenchymatous degeneration of the muscle fibres of the heart, finally passing into a stage of fatty metamorphosis, may occur. The muscle fibres are degenerated, nuclei are broken down or have disappeared, and the fibres themselves cease to be continuous. These alterations

are very marked in the late stages; in the cases which terminate most rapidly only slight parenchymatous changes are apparent.

Sidney Martin [10] published the first of his well-known papers on diphtheritic toxæmia in 1891, in which he dealt with the chemical pathology of this difficult subject. The albumose which he isolated from the tissues in fatal cases of diphtheria and injected into rabbits, was found to produce no effect on the liver, kidneys, spleen and adrenals. Muscle and nerve degeneration occurred. The heart muscle was in a state of fatty degeneration. A few degenerated fibres were present in the vagus in those experimental animals which died during a period varying from seven to eleven days.

Two rabbits received large doses of the albumose, and were killed in 115 days and 94 days respectively. In each instance the heart muscle was very fatty.

He also reports two cases of diphtheria of which he had made a careful pathological investigation.

Case 1.—Child, aged 5, died at the end of the fifth day of disease from diphtheria. The heart muscle was normal and diaphragm likewise.

Right phrenic.—Myelin was broken up and in a few fibres the axis cylinders were ruptured.

Case 2.—Man, aged 19, died from diphtheria on the twenty-seventh day of disease.

Heart muscle.—Few fibres showed fatty change.

Diaphragm.—Some fibres showed marked fatty change, some were only slightly affected, and many were normal.

The right vastus, internus and externus, and similar muscles on the left side, showed scattered fatty change.

The palatal muscles presented a similar appearance.

Nerves to vasti showed degenerative changes, and the phrenics were found to be abnormal.

An animal was killed on the twenty-fourth day subsequent to its receiving a dose of the organic acid, isolated from the diphtheritic toxin by Sidney Martin. The heart muscle showed well-marked fatty change, and the diaphrag also to some extent. The phrenics were normal.

Martin, therefore, concluded from his results that the bacillus of diphtheria forms, both in the body and in culture media, proteid products of the same chemical nature as those found in patients dead from diphtheria—an albumose and an organic acid.

In a second report published in 1892-1893, Sidney Martin [11] stated that all experimental animals which had been injected with the albumose and organic acid show well-marked degeneration of the cardiac muscle, and it was found in rabbits 94 and 115 days after a single injection of the albumose. He considered that the poison acted directly on the cardiac muscle.

This report also contained the result of the examination of three cases of syncope with cardiac failure.

Case 1. — Laryngeal diphtheria. The heart muscle showed diffuse fatty change.

Case 2.—Pharyngeal diphtheria. Diffuse fatty change was present in the heart muscle.

Case 3.—Every fibre of the heart muscle showed marked fatty change. Transverse striation was completely lost.

Finally, he says, "The conclusions to be borne in mind, therefore, are that the signs of cardiac failure in diphtheria are due to a direct effect of the diphtheria poison on the cardiac muscle, and that the fatal syncope, which not unfrequently occurs, is essentially cardiac in origin."

Brewer, of the Homerton Fever Hospital, quoted by Bolton [3], found in thirty-nine consecutive cases of acute diphtheria excessive fatty degeneration in every instance. He also examined eight cases which died late in the disease, and showed that fatty degeneration is much less likely to be seen in these cases than in the acute stages, but that the interstitial changes are liable to be found.

Rabit and Phillippe, also quoted by Bolton [3] in 1891, examined five fatal cases of diphtheria, and found fatty degeneration of the myocardium in the acute stage, but did not consider it to be the cause of recognisable heart symptoms. They believe that the patients die of pure toxæmia, while heart failure during the convalescent stage is due to patchy interstitial myocarditis.

In 1891 Bolton [4] made an exhaustive examination of the neuro-mechanism of the heart in eleven fatal cases of diphtheria. In all instances he found acute degenerative changes in the medulla oblongata, both the sensory and motor nucleus of the vagus being affected. The change apparently begins round the nucleus of the cell in which there is evidence of chromatolysis. This process spreads in every direction throughout the cell, and finally affects the dendrites; later stages are seen in which the whole cell is finely granular and has only a single layer of Nissl bodies arranged around the periphery. At this period the nucleus looks swollen and is usually excentric. At a still later stage there is a further change in the position of the nucleus, which now appears on one side of the wall of the cell, and frequently causes a local bulging of this wall as if it were about to be extended.

In all the eleven cases there was extensive fatty degeneration of the heart. The vagus nerve was normal in every example. Bolton considers that death in acute diphtheria is due to primary heart failure, which can be accounted for by the extensive fatty change which is found in the heart muscle, and the acute degeneration in the motor nucleus of the vagus. He considers that in the less toxic cases fatty degeneration of the cardiac muscle is absent, but that many fibres show cloudy swelling, although, as a whole, these fibres are very small in number in comparison with the normal fibres. The degree of degeneration in the medulla runs *pari passu* with that in the heart and all the acute degenerative changes which occur within the first few days of the disease.

Technique.

Histology.—I will first describe in detail the methods which I have employed in every instance for the purpose of identifying fat in the tissues. The portion of tissue to be examined was placed as soon as possible after death in 10 per cent. formalin in normal salt solution for twenty-four hours, (2) then in running water for twenty-four

hours, and (3) followed by gum arabic for twenty-four hours. The tissue was then cut by the frozen method and the sections were floated out on warm water, and, after a short time, placed in small bottles containing a filtered (just before use) saturated solution of Scharlach R. in 75 per cent. alcohol for twenty-four hours. They were then washed for a few seconds in 70 per cent. alcohol and then in water, and finally mounted in Farrant. In every instance one section of each portion of tissue was counter-stained in Mayer's hæmalum.

Sudan III. was also employed as a fat stain in a similar way to Scharlach R., but the results obtained by the latter method were preferred. It has been recommended by some histologists to stain the sections for ten to twenty minutes in warm Scharlach R. or Sudan III., but I have found this method to be useless for the delicate work such as has been required in the present line of research.

G. Herxheimer recommends a solution of Scharlach R. in alkaline alcohol, or an alcoholic acetone solution. I have given a full trial to the former method, but found it to be extremely unsatisfactory. It acts very well for the coarse fat droplets which give the characteristic feature to adipose tissue, but is of little use for the fine fatty changes met with in diphtheritic toxæmia. It is claimed by Herxheimer that both these methods are rapid; that may be so, but rapid methods which produce negative results are not of much use, and that is my experience of the rapid methods recommended for demonstrating fat in tissues.

Paraffin sections were made in many instances of the viscera both of the infected animals and also of the children which died from diphtheria, and these were stained in various ways. van Gieson's method was found to be the most valuable stain for observing the various changes which may occur in muscle which has been subjected to the action of this powerful toxin.

Busch's modification of the Marchi method was employed for the examination of the heart muscle, diaphragm, vagus, and phrenic nerves. A 1 per cent. and a 0·3 per cent. solution of osmic acid were employed for the same purpose.

In the case of the vagus and phrenic nerves, small pieces were taken from various portions of the nerves along the whole of their course, including the terminal portion. These pieces were placed in Busch's fluid frequently changed for about ten days, and teased preparations were then made and mounted in Farrant.

Portions of the brain were examined by Nissl's method as used in the pathological department at Queen's Square, and also with Leishman's stain employed in various ways.

The toxin.—The diphtheritic toxin which was used in these experiments was obtained from Dr. Cartwright Wood, to whom I am greatly obliged. Virulent and attenuated toxin were employed—but perhaps the most satisfactory results were obtained with the toxin which had been attenuated with anti-toxin. In a few instances in which a large dose of the virulent toxin had been given, more especially intravenously, the animal died without showing some of the more important changes met with under less severe conditions.

A Summary of the Microscopical Appearances in Acute Diphtheritic Toxæmia.

It has long been known that the extra-cellular toxins of the bacillus of diphtheria are capable of producing extensive microscopical changes in various portions of the body, more especially in the heart muscle. This subject has been carefully dealt with by various investigators, but, without doubt, the most valuable papers which have yet appeared on the cardiac changes in diphtheritic toxæmia have been contributed by Dr. Bolton. It is surprising that although so much work has been done on this subject, yet in the standard books on medicine there is little or no reference to those contributions to which I have just previously referred.

There is little doubt that the most important group of symptoms which occur in diphtheria are those which are described as "cardiac paralysis." Most of the previous workers on this subject have studied the changes either in the cardiac muscle, the vagus nerve, or in the vagus

nucleus in the medulla, while in only a few instances investigations have been made of the tissues throughout the body. It is probably owing to this fact that the term "cardiac paralysis" has been invented.

As I have already stated, the diphtheritic toxins show a special affinity for the cells of the heart muscle, which undergo degenerative changes and in which fat droplets accumulate, hence the popular term—fatty degeneration. Bolton has shown that this may occur as early as the third day of disease.

As far as I am aware, all previous investigators on this subject have studied the fatty changes which are produced by these toxins by employing osmic acid. I have used, as already mentioned, Scharlach R. and Sudan III. for this purpose as well as osmic acid and Busch's modification of the Marchi method. There is not the slightest question that for demonstrating these acute fatty changes the red stains are decidedly preferable.

It has been previously mentioned that the fatty changes which are produced in the heart muscle by this toxin have been demonstrated in the human subject as early as the third day. The earliest record among my cases was on the fourth day of disease, and in this instance fatty change in the heart muscle was extremely well shown. In the animal experiments, however, it occurred within *sixteen hours*. In many instances the entire portions of the cardiac muscle which were examined showed diffuse fatty change. In some instances it was more scattered than diffuse, more especially in the hearts of the inoculated animals. The fine fatty change to which I have referred often required very careful examination with the $\frac{1}{12}$th oil immersion before it could be detected, and there is no doubt that in such cases it is advisable not to use a counter-stain, such as hæmalum, but to rely entirely on the fat stain. In a few of the diphtheritic hearts the fatty change which occurred might be described as belonging to the coarse and medium type. In such examples the fat droplets in the muscle cells were of very large dimensions. I was not able in any instance to detect any difference in the microscopical appearances in

the various regions of the heart muscle, as some observers have previously noted. In osmic acid preparations the fatty change was never as well marked as in specimens stained by Scharlach R., while Busch's method was often found to be useless. Granular change was noted in some instances both in the human and experimental cases, but one must be careful to distinguish the normal granularity of the heart muscle, which is a very marked feature, from that which may be regarded as a distinct pathological condition. The muscle cells in which the fatty change could be demonstrated were in some instances very degenerated, while in other examples they appeared to be normal. All intermediate forms between these two extremes were met with. The usual type of degeneration of the cells of the cardiac muscle were as follows: there was loss of transverse striation, but longitudinal striation was more or less well shown; the cell nucleus was either swollen and distorted, or, in some instances, shrunken or completely absent; the cell itself was irregular in shape, while in the most severe cases the entire cell presented a homogeneous appearance. These changes occurred either in cells in which a fatty change could be demonstrated or otherwise. Fragmentation and segmentation were found in some instances, chiefly in the human cases. Vacuolation of the muscle cells was occasionally seen.

Although these diphtheritic hearts show histologically such a very marked fatty change, yet, macroscopically, there is little to detect beyond the fact that they are usually soft and flabby. This fact only shows that it is often impossible to give an accurate opinion of the changes which may be present in the tissues without microscopical examination.

I have failed to find fatty change in the heart muscle in the fourteen control cases of patients dying from various diseases which I examined (see appendix), except in the muscle fibres immediately underlying the inflamed pericardium in cases of pericarditis, and in an example of very rapid death from poisoning by oxalic acid in a woman, aged 40. In this case there was extensive diffuse fatty

change present throughout the cardiac muscle resembling the most acute example of diphtheritic toxæmia. Fat droplets were present, chiefly of the fine type. The muscle fibres appeared to be in good condition, but, in a few instances, the nuclei stained poorly, and the transverse striation was lost. A few cells were completely degenerated.

Well-marked inflammatory reaction of the heart muscle has been noted by the large majority of investigators on this subject, as previously stated. Romberg [16] drew special attention to this feature, but considering that in five out of eight cases pericarditis was found at the autopsy, and in the remainder endocarditis, we must look for some cause other than the diphtheritic toxin for the production of the inflammatory lesions which occurred in his cases.

It is a matter of common knowledge that inflammation of the heart muscle is found, without exception, in all cases of pericarditis and endocarditis, but in diphtheria inflammation of the serous membranes is uncommon.

Hesse [8] also draws special attention to the interstitial myocarditis which he found in his cases.

Flexner [7] is one of the few observers who failed to find any evidence of inflammation of the heart muscle in diphtheritic toxæmia.

In the large majority of my own cases, both the experimental and those in the human subject, there was complete absence of inflammatory processes. In a few instances, however, very slight inflammatory changes were noted. We must be fully alive to the fact that, while the earlier observers considered that diphtheria was a true toxæmia, more recent experience tends to show that in the very acute cases this disease must often be classed as a septicæmia. In such instances the presence of phagocytes in the diseased tissues in which the bacilli are present is only what takes place in every bacterial infection, but in all my *experimental cases* bacterial free toxins were employed, and there was complete absence of inflammatory reaction—as already mentioned—except at the seat of inoculation.

In conclusion, it may be justly said that there are two typical forms of fatty change of the cardiac muscle in acute

toxæmia: (*a*) diffuse and fine fatty change; (*b*) scattered fatty change, showing fine, medium, and coarse droplets.

Diaphragm.

Dr. Caiger tells me that he has never noticed weakness or paralysis of the diaphragm in the *very acute* stages of diphtheritic toxæmia. It is quite evident, therefore, that the action of the diaphragm during the acute stage of the disease is apparently normal.

There is no reference in the literature to the pathological condition of the diaphragm during this acute period of the infection. I have found during this investigation that this muscle shows the acute degenerative changes before the heart muscle, and in some instances diffuse fatty change may occur while the cardiac muscle is apparently normal. This fact in itself serves to prove the most important point in diphtheritic toxæmia, that the toxins act directly on the heart muscle and diaphragm.

In a guinea-pig injected with the diphtheritic toxin, fatty change was detected in the diaphragm within *four hours* from the time of inoculation, that is to say, before any other change had occurred beyond the congestion of the supra-renal glands.

One cc. of diphtheritic toxin was injected subcutaneously into a guinea-pig, and the animal was killed in *four* hours. Supra-renal gland appeared macroscopically to be healthy.

Diaphragm.—Very fine fatty change present in a few of the fibres, nothing else abnormal detected.

Heart muscle.—Normal.

Adrenal gland.—This viscus was found on microscopical examination to be extremely congested, but the fatty change was almost entirely limited to the cortex.

One cc. of diphtheritic toxin was injected into the right leg of a guinea-pig, and the animal was killed in *six* hours. Supra-renal gland was congested.

Heart muscle.—Normal.

Diaphragm.—Fine fat droplets scattered in an irregular manner in the muscular bundles of the diaphragm. No other changes detected.

Adrenal gland.—Abundant evidence of fat in the cortical portion of the gland, and to a less extent in the medulla.

One cc. of diphtheritic toxin was injected into the right leg of a guinea-pig, and the animal was killed in *eight* hours.

Heart.—Normal.

Pancreas.—Normal.

Intercostal muscles.—Normal.

Abdominal muscles.—Normal.

Spleen.—Normal

Diaphragm.—Diffuse fatty change present throughout the muscular tissue. Both fine and medium-sized fat droplets are present. The transverse striation is well marked in some of the fibres, in others absent or deficient. No inflammatory reaction observed.

Liver.—Extreme fatty change present, especially at the periphery of the cells, and between the hepatic cells.

Phrenic nerve.—Normal.

Suprarenal gland.—Cortical portion shows marked fatty change, also present to a less extent in the medulla.

Similar proceedings taken as in the experiment previously referred to, but only 0·5 cc. of toxin was injected. Animal was killed at the end of eight hours.

Phrenic and vagus nerves.—Normal.

Diaphragm.—Very severe fatty change present in most of the fibres; both fine and medium-sized fat droplets present. Some of the muscular bundles appear to consist of little more than fat. No inflammatory reaction observed. Transverse striation in many of the fibres absent, in others feeble, in some it appears to be normal.

Heart muscle.—Normal.

Intercostal muscles.—Normal.

Spleen and pancreas.—Normal.

Kidneys.—Very slight fatty change present in the tubular epithelium.

Liver.—Hepatic cells show abundant cloudy swelling. Very marked fatty change present in the hepatic cells, and also between the liver cells.

Suprarenal gland.—Extensive fatty change present in the cortical portion, and existing throughout the medullary region of the gland.

In the account of the four experiments which are quoted here, marked changes in the diaphragm were found

as early as four, six, and eight hours. The common type of fatty change consisted of very fine fat droplets distributed in patches throughout the muscle fibres. Diffuse fatty change such as occurs in some instances in the heart muscle was never seen, and coarse fat droplets were completely absent. The muscle cells otherwise showed similar degenerative changes to those already described as occurring in the heart muscle.

Inflammatory reaction was present in the case of a child which died with an empyema complicating diphtheria. In this instance there was acute inflammation of the diaphragm immediately beneath the infected pleura. It is quite obvious that the inflammatory reaction was due to the micro-organisms which produced the empyema, and was in no way related to the diphtheritic toxins, as some observers would have us believe.

Skeletal Muscles.

None of the *skeletal muscles*—either in the human or experimental cases—ever showed fatty change. Parenchymatous and granular degeneration were found in some instances.

The leg muscles of the rabbits and guinea-pigs, at the seat of inoculation, presented every variety of degenerative change, except that there was no microscopical evidence of fat.

The inflammatory reaction, here, was extremely well marked. Phagocytes were collected in large numbers among the degenerated muscle fibres, There is no need to credit the diphtheritic toxins with the whole of this reaction. It is well-known that the injection of normal saline will determine the production of large numbers of phagocytes at the seat of inoculation, but as the injection of the diphtheritic toxin consists not only of the toxins themselves, but also of blood serum and broth, there are many factors to account for the inflammatory changes which are produced. It seems likely that the toxins themselves, occurring in a concentrated form, produce necrosis and inflammation of the muscle fibres at the seat of in-

oculation, and degenerative changes elsewhere. It is quite certain, however, that necrosis and inflammation occur at the seat of inoculation and that fatty degeneration is found in more distant parts. I have made a bacteriological examination of the inflamed leg muscles at the seat of inoculation and have recovered the white staphylococcus, but not in a sufficient number of instances to be able to arrive at a definite conclusion.

Stomach and Intestines.

I have failed to demonstrate any fatty change in the involuntary muscles of the stomach or intestines either in the human or experimental cases. I made a very careful examination of the stomach wall in guinea pigs because *acute dilatation* of this viscus is extremely well shown in these animals, which rapidly succumb to the action of the toxin. Villey [18] attached considerable importance to the fatty change which he found in the mucous membrane, and to the round-celled infiltration of the mucosa and submucosa. The round cells formed definite masses in the stomach wall and the cells themselves resembled lymphocytes. When we consider that the fatty change was limited to the mucous coat of the stomach and that the description which is given of the round-celled infiltration accurately corresponds to the normal histological characters of the stomach wall, it is difficult to understand how it would be possible for the changes described by Villey to be in any way related to the vomiting of diphtheria as he has suggested. If the toxins of the bacillus of diphtheria produced an inflammatory reaction such as Villey has described, we should find that the cells present in the inflammatory areas would be the finely granular polynuclear cells, and not cells resembling lymphocytes. This is conclusive evidence that the areas of round-celled infiltration previously referred to are in no way related to the diphtheritic toxin.

Bladder.

No abnormal changes were observed either in the human or experimental cases.

Tongue.

No fatty change was noted in the muscular substance of the tongue in the experimental cases, while in the human cases it was not examined for.

Spleen.

Slight fatty change was detected in a few instances, but it was never well marked. Foci of necrosis with areas of inflammation such as were described by Flexner were never seen.

Pancreas, Thyroid, Thymus and Lymphatic Glands.

Nothing abnormal was detected. It does not appear to be sufficiently recognised that all the blood glands normally contain a considerable quantity of fat, which varies even in the normal gland according to the age of the patient or animal.

Kidneys.

In some instances fatty change was extremely well shown, in most cases it was present to some extent. The fatty change was best observed in the epithelium of the convoluted tubules, but none of the renal epithelium was exempt. Congestion of the kidneys was a prominent feature both in the human and experimental cases. Pathological changes in the vessel walls were not observed. The kidneys were very carefully examined for areas of inflammation, more especially in those instances in which albumen had been found in the urine during life, but in no instance was any inflammatory reaction detected. Other varieties of degenerative changes were also seen in the renal epithelium.

Liver.

This viscus seemed especially prone to suffer from the effects of the diphtheritic toxin. In one case the hepatic tissue consisted of little more than fat droplets, while in most instances fatty change was well marked. In the experimental cases the fat droplets were especially

prominent in the areas surrounding the hepatic veins. It was also found that the peripheral portion of each cell showed fatty change, which was absent or only present to a slight extent in the central region. In some instances the hepatic cells were shrunken and distorted, and the outline of each cell was indistinct or the cells showed degenerative changes. I never noticed any area of inflammation or foci of necrosis.

Adrenal Gland.

The most characteristic lesion produced by the diphtheritic toxins in guinea-pigs is congestion of the adrenal gland. The change can be detected in these animals at the end of four hours after receiving an injection of the toxin, while in later cases the congestion becomes more marked and finally the gland may consist of little more than blood. This condition has been commented upon by most of the previous workers on this subject. Perhaps the most important fact to be noted is that adrenal congestion is always present in these experimental cases; it is surprising, therefore, that it is not more common in the human subject, but one is rather inclined to the view that adrenal hæmorrhage is distinctly rare in diphtheria in children. There is no doubt that adrenal congestion is much more difficult to detect in children than in guinea-pigs, because of the comparative ease with which abnormal colouration of this gland can be observed in these animals. There is one other point to remember, however, that in guinea-pigs which have received antitoxin immediately following the injection of the toxin, adrenal congestion is generally either slight or absent, although the animals may have rapidly succumbed to the disease. This fact may afford some explanation of the rarity of adrenal hæmorrhage in children. In one of my cases the right gland was hæmorrhagic, but there was also a right-sided empyema in this case, which detracts somewhat from the importance of the observation.

It is usually stated in books on histology that the adrenal gland contains some fat which is practically limited to the

outer layer of the cortex. I have now examined a very large number of apparently normal suprarenal glands, both in man and animals, and have been struck by the large amount of fat which is present both in the cortical cells and in the connective tissue framework. In the medulla, however, it is absent. In guinea-pigs which have succumbed to the action of the diphtheritic toxin, the entire cortex of the adrenal glands appears to consist of fat. Every cell which can be defined is filled with fat droplets. Throughout the medulla there are also large areas of fat present. In the glands obtained from children which have succumbed to diphtheria a similar condition is found to be present. It is probable that this circumstance (degeneration of the medullary portion) may afford an additional explanation to account for the severe depression of the entire circulatory system in acute diphtheritic toxæmia. It is hardly necessary to add that hæmorrhages and congestion are very obvious in most instances. It appears, therefore, that the suprarenal gland is one of the viscera which the diphtheritic toxin is especially prone to attack and to produce severe pathological changes in it.

Costal Cartilage.

It is generally supposed that cartilage plays a passive part in septicæmic and toxæmic conditions. I examined the rib cartilage of several guinea-pigs which had succumbed to the action of the diphtheritic toxin, and the cartilages of several control animals. It is well known that the large cells in costal cartilage contain fat. In the toxæmic guinea-pigs, however, the fatty change was most striking. Each cartilage cell contained numerous large and small fat droplets. This abnormal feature was most marked in the large central cells, and only just present in the flattened cells at the extreme periphery. The cartilage cells of the control animals also contained fat, but *microscopically* the amount of fat present in the cartilage cells of the toxæmic animals was far greater than in the normal guinea-pigs. The fact that the diphtheritic toxin exerts its effect over portions of the body which previously had been thought to be exempt in all general infections, is a matter of considerable interest.

The Blood.

This was only examined for evidence of hæmolysis and for the presence of fat in the serum, as the condition of the blood in acute diphtheritic toxæmia is being investigated at the present time by Dr. Dean at St. Thomas's Hospital. Rabbits which were dying from the effects of the toxin were examined, but the blood in all cases failed to show hæmolysis. Similar results were obtained in the human cases, but unfortunately I was unable to examine an example of acute hæmorrhagic diphtheria. No free fat was detected either by the naked eye in centrifugalised blood or microscopically in blood stained by Scharlach R. in either the human or experimental cases.

Pleural Fluid.

Fluid present in the pleural sacs in the guinea-pigs which had died from the effects of the toxin was examined microscopically. Film preparations which were made and stained with Leishman's stain showed that the cells were chiefly endothelial cells, such as may occur in the pleural fluid in cases of cardiac failure in the human subject. This is further proof that the toxins do not, at any rate in the early periods of infection, produce a general inflammatory reaction.

It is clearly shown from a consideration of the above facts, that the diphtheritic toxin produces fatty change in various portions of the body, and that the changes found in the heart and diaphragm are similar to those in the adrenal gland and liver. If this is fully recognised, it will be obvious that it is an error to regard cardiac failure as anything beyond a group of symptoms dependent upon partial or complete loss of function in one viscus.

Brain.

Various portions of the cortex and medulla of the brains of the inoculated rabbits and guinea-pigs were examined by Nissl's method, modified Leishman's method, and they were also stained by the ordinary methods. No abnormal changes were detected, and there was complete absence of congestion of the capillaries, which was such a noticeable feature else-

where throughout the body. In no instance was the brain examined from any of the cases which died of diphtheria, as Bolton has definitely shown that changes do occur in the vagus nucleus in the medulla in acute diphtheria.

Phrenic and vagus nerves.—In many examples these nerves were carefully examined along their entire course by teasing out portions which had been removed from various levels in the neck and thorax, both in the fatal cases of diphtheria and in the inoculated animals. I failed to detect any change in either nerve, even in the portions inserted into heart and diaphragm, in a single instance, in any of the rabbits or guinea-pigs.

Hamilton Wright [20], who strongly supports the nervous theory to account for the acute changes met with in acute beri-beri, has described certain alterations in the nuclei of the neurilemma which are the earliest changes to appear in the nerves, and are found some days before the true Marchi reaction can be detected. To emphasise the views which Wright holds, I can do no better than quote his own words : " When we come to examine the nervous system of a fatal case of acute beri-beri, we find the true explanation of the disease." Then again, " In acute pernicious beri-beri the vagal cardiac terminations bear the brunt of the poison, and this, together with poisoning of the accelerator terminations, soon leads to cardiac exhaustion and death." The very earliest changes in the nerves on which Wright has laid special emphasis are as follows : The termination of the neurons in the heart and as they pass through the deep cardiac plexus, show a change which is profound, but nevertheless it can only be regarded as a pseudo-degeneration. The nuclei of the neurilemma and the surrounding protoplasm is blackened by the osmic acid. There are intensely black dots at the nodes and internodes, but the myelin has not undergone disintegration. Stanley, of Shanghai, as I have previously mentioned, holds an entirely opposite view to Hamilton Wright. He compares acute beri-beri to diphtheria, and attributes the cause of death in both diseases to the direct action of the poison on the heart muscle. I have found in two of my cases of

diphtheria changes in the nuclei of the neurilemma similar to those described by Hamilton Wright in acute beri-beri, but in both instances death occurred after a period of three weeks from the onset of the attack, and in one case the Marchi reaction was also present in both phrenic and vagus nerves. In both the acute and in the experimental cases I have failed to detect any changes in the vagus and phrenic nerves, and all previous workers on this subject appear to have obtained similar results. It is perhaps necessary to add, however, that the nuclei of the neurilemma of both these nerves in the acute cases of diphtheria were often very granular, but how far this is a pathological condition I am unable to say.

The Effects of the Antitoxin in Modifying the Action of the Toxin.

I was anxious to know how far the antitoxin modified the action of the toxins, specially on the heart muscle, so experiments were undertaken to investigate this point. Dr. Caiger has also given me his opinion on this subject. which I will fully quote. It is as follows:—

"I have no hesitation in saying that even in grave faucial attacks of diphtheria, if antitoxin be given in adequate doses within the first forty-eight hours, a fatal issue during the acute phase of the throat illness is extremely rare, but in cases untreated with serum, or in which its administration has been delayed until marked putrefactive changes have commenced in the exudation, death is likely to occur with signs of profound toxæmia somewhere about the end of the first week, if not before. In cases, on the other hand, which have received full doses of antitoxin at an early date, the fatal issue, if not averted altogether, is in most instances delayed until the latter end of the second or third week, and occasionally even later. Under these circumstances the patient succumbs with symptoms which, taken collectively, may be best described by the term 'cardiac failure,' comprising persistent vomiting, feeble, rapid (occasionally slow) and irregular pulse,

restlessness and anuria ; the gradually advancing depression of the heart's action culminating sooner or later in sudden fatal syncope.

"I much prefer the term *cardiac failure* to 'cardiac paralysis,' since although degenerative changes have been found in the vagus and phrenics in some of these cases, it by no means follows that the fatty change in the cardiac muscle, which you have so clearly shown to be present from a very early stage of the attack, may not also be to a large extent responsible for this grave development. The fact that in those comparatively mild attacks in which the only evidence of cardiac depression is to be found in a temporary disproportion between the pulse rate and the temperature, usually attended with feebleness and marked irregularity of pulse (and they constitute the large majority of the cases), the signs are usually accompanied by more or less ventricular dilatation, is, I think, confirmatory of this view. Clinically, all gradations are seen between a condition of comparatively trivial functional disturbance of a temporary character, coming on during the second, third or fourth week of convalescence, and a complete and fatal depression of cardiac function."

In the case of one guinea-pig which received *160 times* the minimum lethal dose of virulent toxin and also 2,000 units of antitoxin at the same time, although the animal was ill for a few days, complete recovery ensued. It was killed twenty-five days later, but nothing abnormal was detected except for a slight fatty change in the adrenals.

Microscopical Examination.

Heart muscle, liver, spleen, kidneys, diaphragm, pectoralis major, soleus muscle, vagus and phrenic nerves appeared to be perfectly normal.

Suprarenal gland. — Very marked fatty change present throughout the cortical and also present to a slight degree in the medullary portion of the gland.

This experiment affords an excellent illustration of what Dr. Caiger has stated, and when we consider the enormous dose of toxin which was administered the result is most striking.

In most of these experiments in which antitoxin was administered, although death may have rapidly supervened, the toxins were found to have produced less effect than in those instances in which no antitoxin was given. Perhaps the most noticeable feature was the diminution of adrenal congestion in all but one experiment, by comparison with those in which the toxin alone had been administered. If the treatment by antitoxin is delayed until the animal is *in extremis*, then it will be found that little or no absorption takes place from the cellular tissues. This fact, no doubt, affords an additional explanation for the reason why antitoxin often completely fails to produce any beneficial effect.

These instances, in which the administration of antitoxin was found to counteract, to some extent, the action of the toxin, are given in full, as they serve to illustrate one of the most important points in this communication.

Experiment 1.—One-twentieth cc. of attenuated diphtheria toxin was injected subcutaneously into a guinea-pig; 2,000 units of antitoxin were given twenty-four hours later, while the animal was very ill. The heart became very rapid, and the animal died in four days from the commencement of the illness.

The suprarenal glands appeared to be healthy, but the heart muscle seemed to be very soft. There was considerable hæmorrhagic œdema at the seat of the injection of the toxin.

Microscopical Examination.

Heart muscle.—Normal.

Diaphragm.—Well-marked fine and medium scattered fatty change present throughout the muscular tissue.

Suprarenal.—Marked fatty change found both in the cortex and in the medulla.

Experiment 2.—One-twentieth cc. of attenuated diphtheritic toxin was injected into a guinea-pig; 2,000 units of antitoxin were given twenty-four hours later. The animal died on the sixth day of the illness from cardiac failure. There was no hæmorrhagic œdema at the seat of inoculation. The suprarenal glands were healthy.

Microscopical Examination.

Heart.—(Scharlach, R.) — Diffuse fatty change scattered throughout the heart muscle.

Diaphragm.—Well-marked fine fatty change present throughout the muscular tissue.

Intercostal muscles appeared to be normal.

Adrenal gland.—Medullary portion practically normal, but a good deal of fatty change in the cortex.

Kidneys.—Slight fatty change detected in a few of the convoluted tubules, otherwise no changes in the kidneys observed.

Rib cartilage.—Fat found to be very abundant in the cartilage cells, especially in those towards the centre, while the outermost limit of cells hardly showed any fat.

Experiment 3.—One-twentieth cc. of attenuated diphtheritic toxin was injected into the right leg of a guinea-pig ; 2,000 units of antitoxin were administered twenty-four hours later. The animal gradually improved and appeared to be perfectly healthy eight days subsequent to the injection of the toxin.

Microscopical Examination (the animal was killed under chloroform).

Heart muscle.—Normal.

Diaphragm.—Very slight fatty change present in a very few of the muscular fibres, but otherwise nothing abnormal detected.

Kidneys.—Many of the convoluted tubules show fatty change in the epithelial cells, but otherwise the renal tissue appears to be perfectly normal.

Adrenal gland.—Extreme fatty change present, chiefly confined to the cortical cells, but also found throughout the medullary portion of the gland. Nothing else abnormal detected.

Experiment 4.—One-fiftieth cc. of attenuated diphtheria toxin was injected into the right leg of a guinea-pig ; 2,000 units of antitoxin were given twenty-four hours later, but the animal was very ill at the time and died during the same night. The suprarenal gland was hæmorrhagic and the heart muscle was soft. There was considerable œdema at the seat of inoculation of the antitoxin, and it appeared doubtful if much of the antitoxin had been absorbed.

Microscopical Examination.

Diaphragm—which was stained with Scharlach R., osmic acid, and with Busch's method, appeared to be normal.

Heart muscle.—Showed a very slight fine fatty change in a few of the fibres by all three methods which were employed in staining the diaphragm.

Suprarenal gland.—Fatty change extremely well marked both in the cortical and also in the medullary portion of the gland.

The pleuritic fluid was examined and the cells which were present were found to be almost entirely endothelial cells.

The Nature of the Fatty Change.

When we consider the rapidity with which the diphtheritic toxins act, especially on tissues of such *vital* importance as the heart, diaphragm, adrenal gland and medulla oblongata, it only shows the truth of Dr. Caiger's remarks and of the absolute necessity of giving the anti-toxin immediately the clinical diagnosis is made. It is difficult to understand the action of the various drugs recommended for the treatment of cardiac failure when the histological characters of the heart muscle in acute toxæmia are recalled. There is one direct line of treatment, and that is due to the immortal work of Professor Behring.

Although at the present day the term "Fatty Degeneration" has almost become obsolete, and has been replaced by "Fatty Change," yet it is probable that when we come to consider the question the original term is not so unscientific as one is led to believe. There does not appear to be any absolute proof that fat can be formed from proteid; it has been frequently stated to occur, but lacks confirmation. It has been shown that bacteria can break down proteid and set free fatty acid. Fresh cheese which yielded 2·16 grm. ether extract, after fourteen days ripening yielded 4·3 grm. Windisch found the ether extract of Camembert cheese increased from 49·78 to 56·75 per cent. in the process of ripening. Because fungi have the power of forming fatty acids and fats out of proteid it does not prove that the same thing holds good for the cells of the animal body, but it at least renders it more probable (Leonard Hill). Other facts which give support to the view that fat can be formed from proteid, is that glycogen can be formed from proteid, and that fat can be formed from carbohydrate. True experimental evidence of this physiological process, *i.e.*, conversion of proteid into fat, has, therefore, yet to be produced.

It has long been known that a large amount of fat can be demonstrated in the viscera in certain diseases by microscopical tests. It was this circumstance which led Virchow to believe that this change was due to the conversion of proteid (protoplasm) into fat. The liver in phosphorous poisoning was considered to be the best illustration

of this remarkable change. Rosenfeld has shown, however, that the fatty change is not due to a fatty degeneration of protoplasm, but to a storage of food fat, and considers it a fatty infiltration. Rosenfeld has also proved that we must not rely entirely on the microscope in these investigations if we wish to obtain accurate results. It is now known that degenerated nerves contain less fat than normal nerves, although by microscopical tests alone one would expect the exact opposite. Rosenfeld proved that in a patch of yellow softening in the brain there was 6·17 per cent. fat, while normally in the same region he found 8·81 per cent. Physiologists have obtained 12·16 per cent. of fat in normal heart muscles. Dr. Leathes has found 11·0 per cent. in the normal diaphragm of the rabbit and 10·6 per cent. in the red muscles of the same animal, yet there is no evidence of fat microscopically. Leonard Hill [9] argues on these grounds "that microscopical evidences of fatty degeneration are utterly untrustworthy." No one would suggest that the microscope could be used as a means for estimating the amount of fat present in a diseased organ, but to my mind we can prove by the aid of the microscope that the tissues have lost their power of using up the fat which is normally present, and the very fact that fatty change can be demonstrated in the cells of the heart muscle is proof positive that these cells are in an abnormal condition, but that is as far as histologists can say. It is hardly possible, however, that the most ardent upholder of the value of physiological chemistry would deny the absolute importance of histological methods except for the true estimation of the amount of fat which is present. It must be pointed out that there are errors on the chemical side. It is absolutely impossible for any physiologist to give an accurate estimate of the fat present in the heart, diaphragm, &c., because there is always a large amount of fat present in the connective tissue which could not possibly be removed by hand while cleaning the muscle fibres, as it cannot be seen except by the aid of the microscope. As in these areas fat droplets are much larger than any we ever see in degenerated heart muscle, the amount of fat

which physiologists have stated to be present must be far in excess of the correct estimate. If, however, the total estimate of fat in healthy viscera is simply given for comparison with diseased organs, then it would be *approximately* correct. In these experiments of mine, the total quantity of fat present in the diseased and normal hearts was made by Dr. Leathes. Dr. Leathes has kindly sent em the report of his investigation, which is given below.

"The hearts were washed free of blood and clots, and all above the auriculoventricular groove was cut away (*i.e.*, auricles, valves, &c.); they were then dried with filter paper and weighed.

Six normal hearts	=	7·38 g.
Six poisoned hearts	=	12·42 g.

They were then carefully cleansed from all visible epicardial and endocardial fat, coronary vessels and, in the case of the poisoned hearts, hæmorrhagic parts about the base of the ventricles were excised, which reduced the weight to 6·54 and 10·94 respectively. They were then dried and powdered.

Dry solids from	6·54 g. normal hearts	= 1,406 g.	= 21·49 per cent.
,, ,,	,, 10·94 g. poisoned hearts	= 2,000 g.	= 18·27 ,, ,,

The dry powder was boiled with alcohol and extracted with chloroform in a soxhlet for six hours, and both processes repeated, as in Rosenfeld's method of fat estimation. The alcohol and chloroform extracts were taken up in ether, the ether solution filtered, evaporated and dried at 105° C.

	Per cent. of fresh tissue.	Per cent. of dry tissue.
Ether extract from normal hearts, 0,2451	3·74	17·43
,, ,, ,, poisoned hearts, 0,3954	3·61	19·77

These extracts were heated with alcoholic potash to saponify the fat, and then dissolved in a large volume of water; the solution acidified with sulphuric acid and kept hot till the insoluble fatty acids had completely separated to the top. These were then filtered off, dried on the filter, and dissolved by extracting the filter in a soxhlet with

petroleum ether, and finally filtered, evaporated and dried. As is always the case with the heart and other organs, the ether extract obtained by Rosenfeld's method yielded considerably less insoluble higher fatty acids than true fat should theoretically yield.

	Per cent. of fresh tissue.		Per cent. of dry tissue.
Insoluble fatty acids from normal hearts	0,1344 = 2·05	..	9·55
,, ,, ,, poisoned hearts	0,2440 = 2·23	..	12·20

The surest index of the amount of fat in a tissue is obtained by the estimation of the fatty acids insoluble in water obtained by saponification of the ether extract. In this way we found that the normal hearts in which no fat can be detected histologically contained 80 per cent. of the amount present in the hearts which showed the most intense histological reaction for fat owing to the action of the toxin. It is clear that the increased amount of fat in the latter is not sufficient by itself to account for the difference in the histological reaction of the two sets of hearts. And the fact that as much as 10 per cent. of the total solids of the normal muscle is fat, and yet none of this fat microscopically is demonstrable, suggests that the real nature of the change set up by the poison consists in the setting free of fat, or some similarly simple compound of the fatty acids, from complex combinations in which these acids normally occur, and in which they are combined in such a way as not to react as they do in the simple fats. The myeline of a normal nerve contains complex compounds of fatty acids which, when fresh or fixed, do not betray their fatty nature. In a nerve that has degenerated as a result of section, these complex combinations break down, and the fat reactions are then obtained, although in this case there is actually less fat in the degenerated nerve than in the healthy nerve from the other side of the body. It seems necessary to suppose that a similar change has occurred in the hearts that had been acted on by the toxin.

It is true that such complex combinations of fatty acids have not been isolated from the heart muscle as they have from nerve tissue. But the normal heart tissue clearly contains a considerable amount of fat that is not histo-

logically recognisable, and the most obvious inference is that it is not recognisable because the unsaturated valencies of the carbon atoms in the oleic acid, to which the histological reactions of fat are due, are not free to react any more than they are when fat is combined with galactose, cerebrines, &c., to form myeline.

This unmasking of fat by the action of the toxin, however, will not account for any increase in the fat, as shown by analysis, and therefore does not account for the whole of the change. This increase, like that due to phosphorus poisoning, we must suppose to be due to an accumulation of imported fat. In every case that has hitherto been investigated in which the fat in a tissue is increased, this increase has been shown to be composed of imported fat. And if there is an accumulation of imported fat as a result of the action of the toxin, the most obvious inference in this case is that the fat has accumulated, not because more has been imported than should have been, but because the muscle cells have been rendered by the toxin incapable of making use of the fat that is brought to them. The toxin has put a stop to the chemical change by which the fat is normally oxidised in the cell, and its action may, therefore, be regarded provisionally as an instance of anticatalytic action.

The other changes which, according to the figures quoted here, appear to result from the action of the poison, are hypertrophic enlargement and an increase in the amount of water in the tissue. In the fresh condition the poisoned hearts were nearly 70 per cent. heavier than those from the normal animals; but the dry solids obtained from them were only about 40 per cent. heavier." (J. B. L.)

Selection of Fat Stains.

There is a point to which I wish to refer, and that is to emphasise the superiority of Scharlach R. (azo-orthotoluol-azo-B-naphthol) and Sudan III. (azo-benzine-azo-B-naphthol) over osmic acid. Mr. Shattock and others have done so previously, but in this research it was found to be most striking. Although all previous workers have employed

osmic acid and some have obtained excellent results, yet, if we study the literature carefully, there is obviously a degree of uncertainty about the results obtained with osmic acid in many instances. It is a matter of common knowledge that osmic acid will stain "droplets" dark brown or green which are not really fat, and this may at times lead to considerable difficulty. The very fine fatty changes which were present in the heart muscle and diaphragm in some examples were not detected with osmic acid, although excellent results were obtained with Scharlach R. While in a few cases, although the very fine fat droplets were stained black, it required considerable care to demonstrate their presence in the tissues. Busch's method is excellent for the nervous system, but I found it to be of little or no value for acute fatty changes in the heart and diaphragm. I am unable to find a solitary point in favour of osmic acid, or even to show its equality with the red stains for demonstrating acute fatty changes.

Doubt has been cast as to whether Scharlach R. and Sudan III. do not stain substances other than fat, simply because it is possible to obtain results with them which cannot be obtained with osmic acid. It is impossible, however, at present, to decide this point. I have made numerous investigations with these stains and the various fats and fatty acids, and see no reason to doubt, at the present time, the value of the red stains.

Summary of my Observations on the Changes produced in the Tissues by the Toxins of the Bacillus of Diphtheria.

(*a*) That the most important lesion in the acute cases is a fatty change of the heart muscle and diaphragm, which is due to a direct action of the toxins on these tissues.

(*b*) That similar fatty changes may be found in certain of the important viscera, more especially the adrenal gland and liver.

(*c*) That the expression "cardiac paralysis" in acute diphtheritic toxæmia should be abolished and be replaced by "acute cardiac failure."

(*d*) That the changes found in the nervous system are secondary factors and not the primary cause of the cardiac failure.

(*e*) That the antitoxin, if given in sufficient quantity and within the first forty-eight hours, may prevent, or at any rate will considerably diminish, the possibility of death from cardiac failure.

In conclusion, my thanks are due to large numbers of my friends for help and advice, but I must especially mention Dr. Foord Caiger. He suggested to me that I should undertake this investigation, he supplied me with the most valuable material, and he placed his vast clinical experience at my disposal. It is impossible for me, therefore, sufficiently to thank him for his invaluable help and kindly criticism. I have also to thank Dr. Sharkey, Dr. Acland, Dr. Hawkins, and Dr. Hector Mackenzie for permission to make use of their cases, Dr. Leathes for much valuable help, and Drs. Box, Harwood-Yarred, Mavrogordato, and H. R. Dean, for the trouble to which they have been put for my benefit on numerous occasions.

Appendix of Certain of the Cases, other than Diphtheria, which have been used for Control Purposes.

Case 1.—Suppurative spinal meningitis and myelitis.

Microscopical Examination.

Heart and diaphragm.—Normal.

Case 2.—Child, aged 2 years, suffering from suppurative osteomyelitis, died, after receiving a few whiffs of chloroform.

Autopsy.—Typical pyæmia. Suppurative pericarditis and myocarditis. The whole of the heart substance was riddled with diffusely spreading abscesses. The heart was sent to me by Dr. H. R. Dean.

Microscopical Examination of Heart Muscle.

Very extensive areas of inflammatory tissue present, also large clumps of Gram positive staphylococci. The muscle cells immediately beneath the *acutely inflamed* pericardium show some fine fatty change, but not elsewhere.

Case 3.—Hypertrophy of the thymus gland in a young child, which was said to have died from lymphatism.

Microscopical Examination.

Heart muscle.—Normal.

Case 4.—Alcoholic neuritis. The vagus, phrenic and recurrent laryngeal nerves, also the heart and diaphragm, were taken for microscopical examination.

Microscopical Examination.

Heart, diaphragm and crico-thyroid muscles.—Normal.

Vagus and phrenic nerves.—Both nerves showed a well-marked Marchi reaction, both at the termination of the nerves in their respective muscles, and also along the whole course of the nerves. The recurrent laryngeal nerve showed an extremely well-marked Marchi reaction.

Case 5.—Alcoholic woman, who died from a perforated gastric ulcer. The heart, at the autopsy, was considered to be a typical example of fatty degeneration.

Microscopical Examination.

Heart.—No fat present. Abundant central pigmentation of the muscle fibres. Fragmentation extremely well shown.

Case 6.—Septic scarlet fever.

Microscopical Examination.

Heart.—Normal.

Diaphragm.—Fatty change present to a high degree in a few of the fibres.

Liver.—Slight amount of fatty change present. All cells in good condition.

Pectoralis major.—Normal.

Spleen.—Normal.

Adrenal gland.—No fat observed in the medulla, but large amount present in the cortical portion of the gland.

Case 7.—Septic scarlet fever.

Microscopical Examination.

Heart muscle found to be normal.

Case 8.—Case of measles, which was admitted to the hospital on February 16, 1905, died March 7, 1905. At the autopsy diffuse broncho-pneumonia was found to be present.

Microscopical Examination.

Heart and diaphragm.—No fatty change detected. (Scharlach R.)

Kidneys.—Very marked swelling of the renal epithelium, but no fatty change present.

Suprarenal.—No fat present in the medullary portion of the gland, but very abundant in the cortical region.

Appendix of Certain Experiments with Diphtheritic Toxin.

Experiment 1.—Fresh heart muscle of a guinea-pig was placed in diphtheria toxin for forty-eight hours, at 37° C., under strict aseptic precautions. Sections were then stained with Scharlach R.

Microscopical Examination.

Heart muscle.—No fatty change detected.

Experiment 2.—Heart muscle treated in similar way for seventy-two hours.

Microscopical Examination.

Heart muscle.—No fatty change detected.

Experiment 3.—Heart muscle treated in similar way for ninety-six hours.

Microscopical Examination.

Heart Muscle.—No fatty change detected.

Experiment 5.—One-tenth cc. of diphtheria toxin injected subcutaneously into a guinea-pig. The animal died in twenty-four hours.

Microscopical Examination.

Heart.—Very fine diffuse fatty change present.

Diaphragm.—Similar to heart.

Leg muscle and intercostal muscles.—Normal.

Phrenic and Vagus nerves.—Normal.

Rib cartilage.—Fat very abundant in the central cells and gradually diminishing in amount towards the periphery.

Adrenal.—Very abundant fatty change observed in the cortex, slight but distinct change in the medulla.

Kidneys.—Very marked fatty change detected in the convoluted tubules, but none observed in the glomeruli.

Liver.—Very marked fatty change present.

Experiment 6.—One-tenth cc. of diphtheria toxin was injected into the left leg of a guinea-pig. The animal died in fifteen hours.

Microscopical Examination.

Heart muscle.—Very fine scattered fatty change found to be present. Very marked granular change observed in the muscle cells. Extensive congestion present. Nothing else abnormal detected.

Leg muscle.—Extensive inflammatory reaction present at seat of inoculation.

Diaphragm.—Similar changes to those found in the heart muscle were noted, except that the fatty change was more scattered.

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Description of Coloured Plate.

Fig. 1.

Section of heart muscle of a child showing diffuse and fine fatty change in muscle cells. Case was fatal on the eighth day of disease.
Stained with Scharlach R. and hæmalum. ($\frac{1}{6}$ obj. and "B" eyepiece.)

Fig. 2.

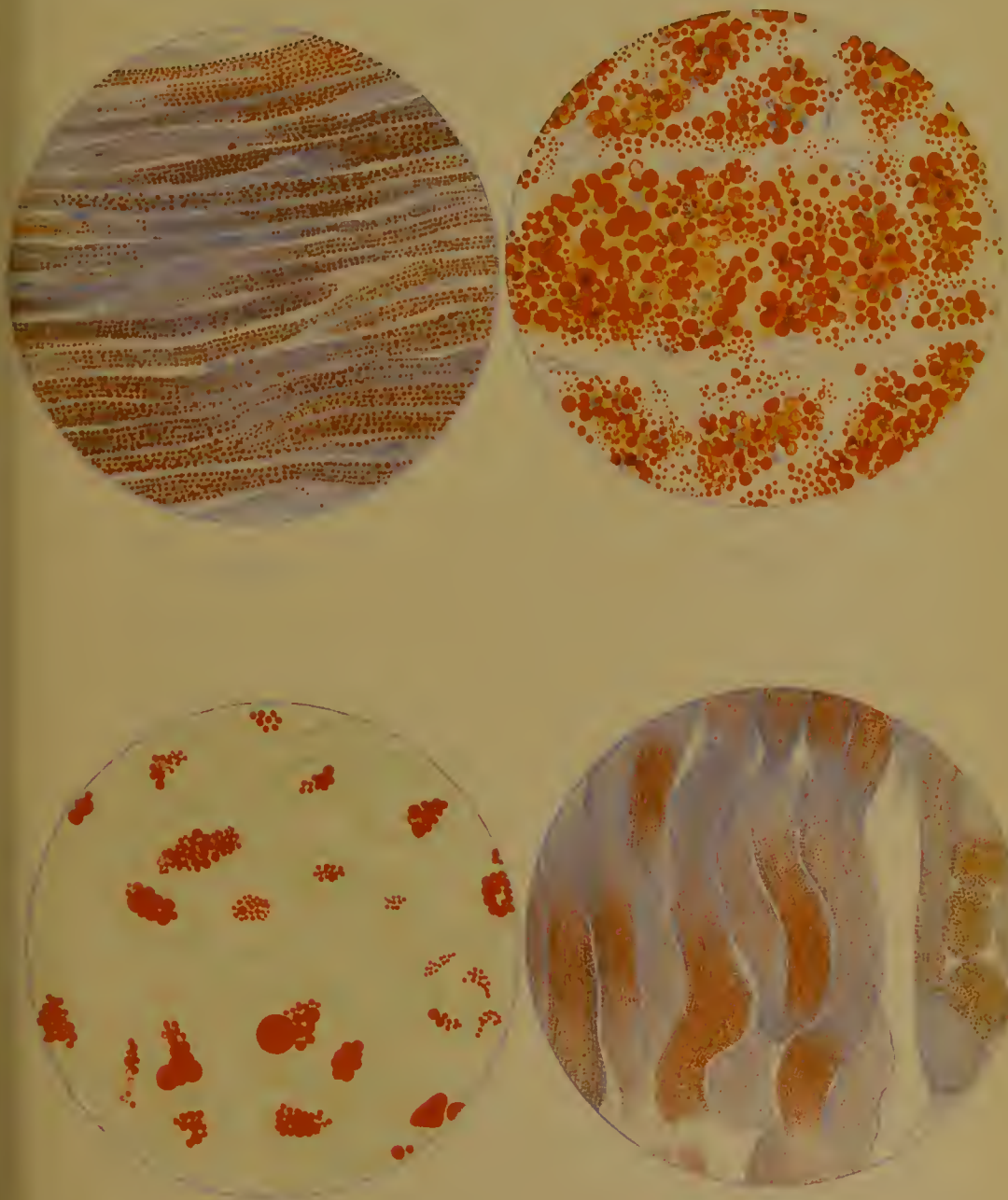
Section of adrenal gland of a guinea-pig which died in 24 hours from acute diphtheritic toxæmia, showing fatty change in the cortex and medulla of the gland.
Stained with Scharlach R. and hæmalum. ($\frac{1}{6}$ obj. and "B" eyepiece.)

Fig. 3.

Section of the costal cartilage of a guinea-pig which died in 24 hours from acute diphtheritic toxæmia, showing extensive fatty change in cartilage cells.
Stained with Scharlach R. and hæmalum. ($\frac{1}{6}$ obj. and "B" eyepiece.)

Fig. 4.

Section of the diaphragm of a rabbit which died in 12 hours from acute diphtheritic toxæmia. Specimen shows fine fatty change in the muscle cells.
Stained with Scharlach R. and hæmalum. ($\frac{1}{6}$ obj. and "B" eyepiece.)



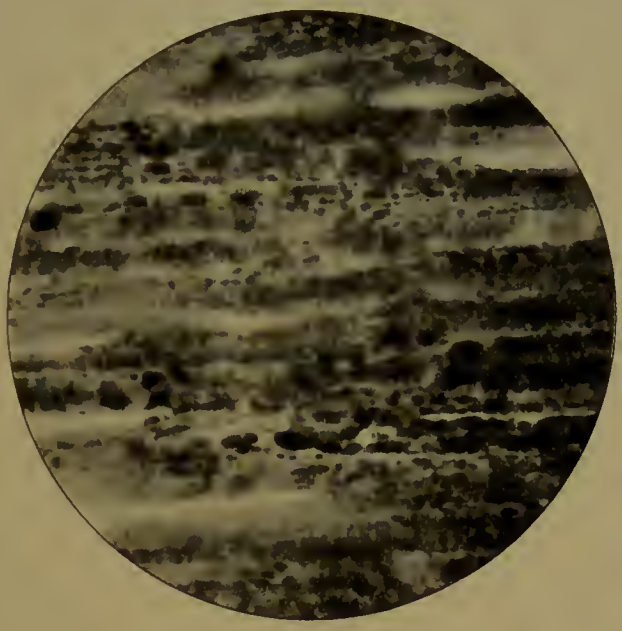


FIG. 5.

Section of heart muscle from a case of diphtheria, fatal on the eighth day of disease.

Specimen shows diffuse and coarse fatty change.

Stained with Scharlach R.

("B" eyepiece and $\frac{2}{3}$ obj.)

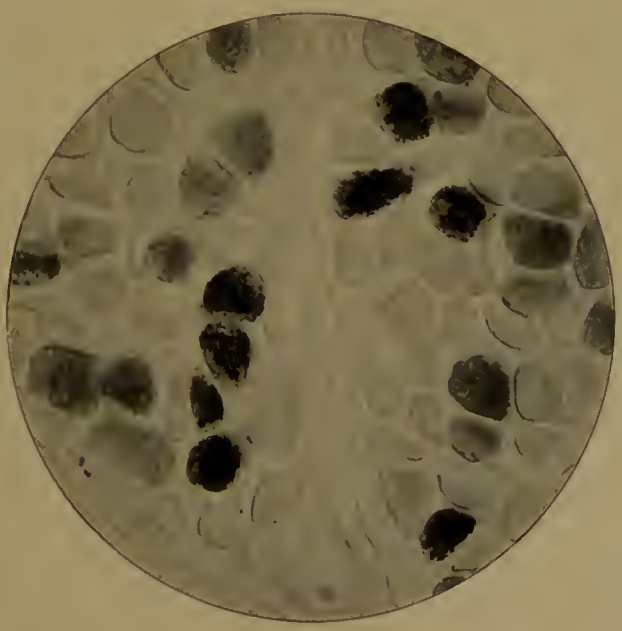


FIG. 6.

Section of human diaphragm showing scattered fine fatty change in the muscle bundles; obtained from a case of diphtheria which was fatal on the fourth day.

Stained with Scharlach R.

("B" eyepiece and $\frac{2}{3}$ obj.)

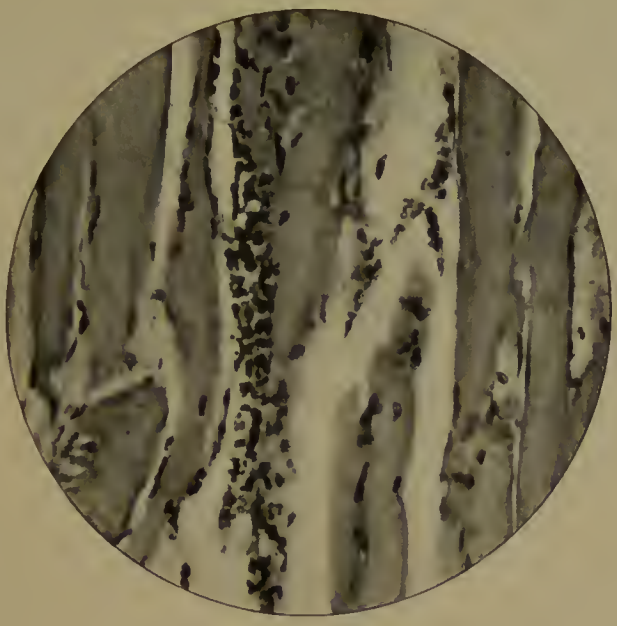


FIG. 7.

Section showing phagocytic invasion of the leg muscles of a rabbit at the seat of inoculation with the diphtheritic toxin.

Stained with hæmalum and Van Gieson's piero-fuchsin.

("B" eyepiece $\frac{2}{3}$ obj.)

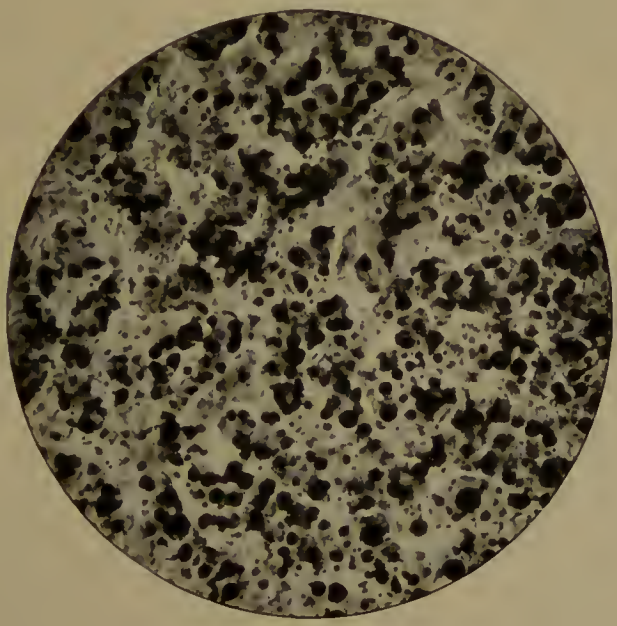


FIG. 8.

Section of human liver obtained from a case of diphtheria which died on the sixth day of disease, showing diffuse fatty change in the liver cells.

Stained with Scharlach R.

("B" eyepiece and $\frac{2}{3}$ obj.)

20cm
32
64
10
16
STAINLESS STEEL
24ColorCard
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